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Published in:
Journal of Thrombosis and Haemostasis

DOI:
[10.1111/jth.14020](https://doi.org/10.1111/jth.14020)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Lisman, J., Bos, S., & Intagliata, N. M. (2018). Mechanisms of enhanced thrombin generating capacity in patients with cirrhosis. *Journal of Thrombosis and Haemostasis*, 16(6), 1128-1131.
<https://doi.org/10.1111/jth.14020>

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COMMENTARY

Mechanisms of enhanced thrombin-generating capacity in patients with cirrhosis

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To cite this article: Lisman T, Bos S, Intagliata NM. Mechanisms of enhanced thrombin-generating capacity in patients with cirrhosis. *J Thromb Haemost* 2018; **16**: 1128–31.

See also Sinegre T, Duron C, Lecompte T, Pereira B, Massoulier S, Lamblin G, Abergel A, Lebreton A. Increased factor VIII plays a significant role in plasma hypercoagulability phenotype of patients with cirrhosis. This issue, pp 1132–40.

The liver is the site of synthesis of many proteins involved in hemostasis, including procoagulant and anticoagulant proteins, profibrinolytic and antifibrinolytic proteins, and thrombopoietin. Consequently, patients with liver disease acquire complex changes within their hemostatic system [1]. Historically, these changes were thought to result in a bleeding diathesis. Although bleeding complications are common in patients with chronic liver disease, the most common bleeding complication, variceal bleeding, is a consequence of portal hypertension, rather than hemostatic failure. Notably, the severity and outcome of variceal bleeding were not different between patients with cirrhosis who did or did not use anticoagulant drugs at the time of the bleed, confirming that variceal bleeding is unrelated to hemostatic failure [2]. Severe bleeding was previously common during large invasive procedures such as liver transplant surgery. However, improvements in perioperative management have led to a substantial decline in bleeding severity during the transplant procedure. Now, a substantial proportion of patients undergo transplant surgery without the requirement for any perioperative blood product transfusion [3]. These clinical observations, combined with laboratory analyses of global hemostatic capacity, have led to the concept of rebalanced hemostasis [4]. Patients with liver disease appear to

have a reset in their hemostatic balance, owing to simultaneous declines in procoagulant and anticoagulant drivers. This new hemostatic balance, however, is much more fragile than the balance in healthy individuals, and may abruptly tip to either a hypocoagulable or hypercoagulable phenotype.

Although, historically, bleeding was a major concern in patients with liver disease, the focus has now shifted to thrombotic complications. Recent studies, including meta-analyses, have demonstrated that chronic liver diseases constitute a risk factor for venous thrombosis [5]. However, the prevention and treatment of venous thrombosis are complex, as it is not established how to best anticoagulate a patient with a profoundly altered hemostatic system at baseline [6]. Another frequent thrombotic complication in patients with advanced chronic liver disease is portal vein thrombosis (PVT). PVT in the patient with advanced cirrhosis is often discovered incidentally during imaging. There is debate about whether PVT is simply a reflection of the severity of liver disease [7], or whether PVT drives progression of disease [8]. Consequently, it is unclear whether treatment of asymptomatic PVT is required. Notably, some of the asymptomatic thrombi resolve spontaneously [7], and prolonged anticoagulant treatment leads to recanalization in only a proportion of patients [9]. A single small randomized controlled trial has demonstrated that prophylactic low molecular weight heparin (LMWH) safely and effectively prevents the development of PVT in a very select group of patients with cirrhosis [10]. Interestingly, LMWH also decreased the rate of decompensation and death, indicating that anticoagulant therapy might delay disease progression, and that prevention rather than (considering) treatment of PVT should be the focus of treatment paradigms.

The concept of rebalanced hemostasis states that all components of hemostasis are in a new balance, with

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Received: 19 March 2018

Manuscript handled by: P. H. Reitsma

Final decision: P. H. Reitsma, 26 March 2018

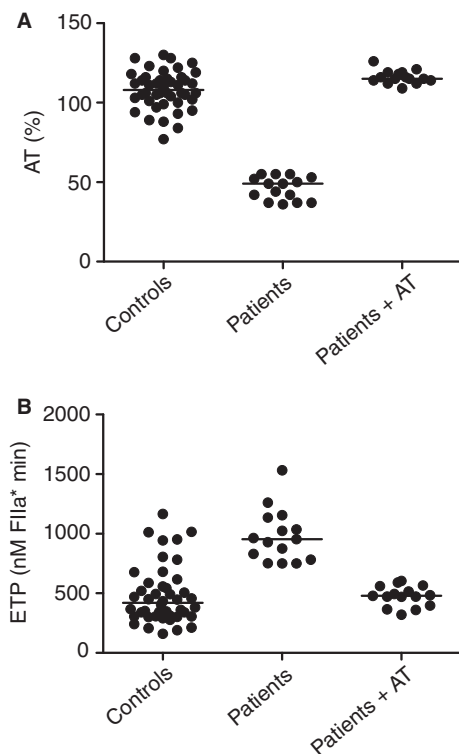


Fig. 1. Effect of normalization of antithrombin (AT) plasma levels on thrombomodulin-modified thrombin generation in plasma from patients with cirrhosis. (A) AT plasma levels were measured on an automated coagulation analyzer (ACL 300 TOP) with reagents and protocols from the manufacturer (Werfen, Breda, the Netherlands) in 15 patients with cirrhosis (six related to alcohol abuse, four to non-alcoholic steatohepatitis, four to viral hepatitis, and one to primary sclerosing cholangitis, with a median model of end-stage liver disease score of 10, range 7–18) and 44 healthy controls. Patients and controls provided informed consent, and the study protocol was approved by the medical ethical committee of the University Medical Center Groningen. In the patient samples, AT levels were normalized by addition of AT concentrate (Shire, Lexington, MA, USA), and plasma levels of AT were reassessed. (B) Thrombomodulin-modified thrombin generation assays were performed on citrated platelet-poor plasma by the use of calibrated automated thrombinography with reagents and protocols from the manufacturer (Thrombinoscope, Maastricht, The Netherlands) in healthy controls, patients with cirrhosis, and patient samples in which AT levels were normalized. Horizontal lines indicate medians.

thrombocytopenia balanced by elevated plasma levels of von Willebrand factor, decreased plasma levels of procoagulants balanced by decreased levels of anticoagulants, and decreased plasma levels of antifibrinolytics balanced by decreased plasma levels of plasminogen (summarized in [1]). However, upon careful inspection, the hemostatic system of a patient with advanced chronic liver disease (i.e. cirrhosis) has clear hypercoagulable features. Such prothrombotic features include an imbalance in the von Willebrand factor–ADAMTS-13 axis, platelet hyperfunction, elevated levels of intravascular tissue factor, and decreased permeability of the fibrin clot (summarized in [11]). Furthermore, thrombin-generating capacity is

elevated when it is tested with thrombomodulin-modified thrombin generation testing.

In 2005, it was first demonstrated that the thrombin-generating system in patients with cirrhosis is much more competent than expected on the basis of diagnostic tests of hemostasis [12]. The prothrombin time and activated partial thromboplastin time are prolonged in patients with cirrhosis, suggesting a hypocoagulable state. However, these diagnostic tests are only sensitive for procoagulant proteins, and are unrelated to hemostatic capacity in patients with disorders in both procoagulant and anticoagulant proteins. Thrombin generation tests, such as calibrated automated thrombinography, are sensitive for anticoagulant systems, but a full representation of hemostatic balance between procoagulant and anticoagulant proteins is only obtained when the thrombin generation test is modified to allow for activation of the protein C system, e.g. by the addition of soluble thrombomodulin to the test mixture. Although the initial article in 2005 demonstrated similar thrombin-generating capacity between healthy individuals and patients with cirrhosis in thrombomodulin-modified thrombin generation tests [12], subsequent studies by multiple independent laboratories have actually demonstrated increased thrombin generation in patients [13–16].

An article by Sinegre *et al.* in this issue of the *Journal of Thrombosis and Haemostasis* addresses the question of which factors determine enhanced thrombin-generating capacity in patients with cirrhosis [13]. Previous studies have demonstrated that regulation of thrombin generation by the protein C pathway is decreased in patients with cirrhosis, and that normal to increased thrombin generation in thrombomodulin-modified thrombin generation tests relates to resistance to the anticoagulant action of thrombomodulin [17]. This thrombomodulin resistance is a result of both decreased plasma levels of protein C and increased plasma levels of factor VIII [18,19]. Recently, the ratio between the results of thrombin generation tests performed in the absence and in the presence of thrombomodulin, or the ratio between FVIII and protein C levels, have been used to indicate a hypercoagulable status in patients with cirrhosis [17,20]. However, as these ratios are not necessarily related to overall thrombin-generating capacity, it may be more appropriate to assess the results of the thrombomodulin-modified thrombin generation test in determining the hemostatic capacity of plasma from patients with cirrhosis. Sinegre *et al.* did exactly this, and demonstrated increased thrombin generation in patients with cirrhosis as compared with healthy controls, with thrombin-generating capacity increasing with increasing disease severity. The authors addressed the roles of elevated FVIII and decreased protein C levels in the hypercoagulable state of patients with cirrhosis by normalizing plasma levels in individual patient samples. Protein C levels were normalized by the addition of purified protein C, and FVIII levels were normalized by a

very elegant approach in which some of the plasma FVIII was inactivated by an antibody against FVIII, which was titrated so that residual FVIII activity was close to 100%. The authors showed that the increased thrombin generation in patients was only partly normalized when only protein C or only FVIII was normalized, but full normalization occurred when protein C and FVIII levels were normalized simultaneously. The authors carefully conclude that enhanced thrombin generation in patients with cirrhosis is explained by opposite changes in plasma levels of FVIII and protein C. Although high FVIII and low protein C levels clearly contribute to enhanced thrombin generation in patients with cirrhosis, this is probably only part of the story.

Thrombin-generating capacity is the net result of plasma levels of procoagulant and anticoagulant proteins, all of which (perhaps except for levels of tissue factor pathway inhibitor) are altered in patients with cirrhosis. To demonstrate that high FVIII and low protein C levels are not the only contributors to enhanced thrombin generation in patients with cirrhosis, we studied thrombomodulin-modified thrombin generation in 15 patients with cirrhosis and decreased antithrombin plasma levels before and after normalization of antithrombin levels. As shown in Fig. 1A, antithrombin levels were substantially decreased in patients as compared with 44 healthy individuals, and levels were normalized after *in vitro* addition of antithrombin concentrate. Figure 1B shows elevated thrombin generation in patients as compared with controls, which was fully normalized when antithrombin plasma levels were normalized. These data are almost identical to the data in Figure 4 of the Sinégre *et al.* article, and illustrate that enhanced thrombin generation in cirrhosis is the result of multiple complex changes in the plasma levels of procoagulant and anticoagulant proteins that not limited to high FVIII and low protein C levels.

Thus, Sinégre *et al.* confirm and extend previous findings on the mechanisms underlying enhanced thrombin generation in plasma from patients with cirrhosis by showing that not only low protein C levels [18], but also high FVIII levels contribute to the hypercoagulable state. However, as the coagulation potential in cirrhosis depends on the interaction between all procoagulants and anticoagulants, isolated assessment of FVIII or protein C levels in these patients yields incomplete information. Rather, global tests such as thrombomodulin-modified thrombin generation should be used to estimate the hemostatic status of these patients.

Notwithstanding the complexity of the mechanisms underlying the enhanced *in vitro* thrombin generation in patients with cirrhosis, the data of Sinégre *et al.* help us to better understand the mechanisms underlying this particular hypercoagulable feature in the hemostatic system with complex alterations in patients with cirrhosis. Future studies will have to address whether enhanced thrombin generation in patients with cirrhosis contributes to the

elevated risk of important clinical outcomes, such as venous thrombosis and/or PVT, and what steps should be taken to safely neutralize this hypercoagulable phenotype in order to avoid these potentially devastating thrombotic events in this patient population.

Addendum

T. Lisman drafted the manuscript and supervised the experiment. S. Bos and N. M. Intagliata provided intellectual input and commented on the initial draft. S. Bos included patients and controls.

Acknowledgements

The authors thank J. Adelmeijer for expert technical assistance.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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